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Patents ADP number (if you know it) 8571051001

If the applicant is a corporate body, give the country/state of its incorporation United Kingdom

4. Title of the invention Stable Integrands

5. Name of your agent (if you have one)
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STABLE INTEGRANDS

The present invention relates to transposable elements and methods for their incorporation into the genome.

Transposable element technology has enabled the genetic transformation of a wide range of insects [*c.f.* Handler, A. (2001), Insect Biochem Mol. Biol. *31*, 111-128; Handler, A. (2002), Insect Biochem. Mol. Biol. *32*, 1211-1220; Horn, *et al.*, (2002), Insect Biochem. Mol. Biol. *32*, 1221-1235], and at least some of these transposable elements have also been shown to be mobile over a much wider phylogenetic range, including prokaryotes and vertebrates (Handler, 2001, *supra*).

Transposons have been described extensively in the prior art. The elements used for genetic transformation of insects are generally characterised by opposing inverted repeat sequences and are associated with an optionally integral transposase enzyme. The transposase recognises the inverted repeat sequence and excises the sequences, together with any intervening DNA, and then reinserts the resulting transposon at another site, either in the genome or in a plasmid.

An autonomous transposon encodes its own transposase in the DNA between the inverted repeat sequences. Such autonomous transposons are not stably incorporated into the genome, as they are liable to move at random. Accordingly, where such a transposon is to be used to incorporate target DNA into a genome, it is preferred to provide the transposase on a helper plasmid, for example, so that transposition is dependent on the availability of the helper plasmid.

This system for incorporating genes or nucleic acid sequences into genomes is effective, but the transposed DNA is still associated with the inverted repeat sequences of the transposon, so that any future exposure to transposase can result in movement of the transposon, possibly even across the species barrier, although this unlikely.

The presence, or potential presence, of transposases capable of remobilising these insertions has led to objections to the use of this technology, particularly when the transformed organisms are for release into the environment. In addition, the presence of such transposases will tend to destabilise the insertions, which is generally undesirable. Though laboratory strains

can be screened for the presence of related transposases by any of several methods, based, for example, on nucleic acid sequence similarity, such as by hybridisation or PCR, or function, such as plasmid-to-plasmid transposition assays or plasmid excision assays, it is not possible exhaustively to test all wild populations.

It is therefore desirable to develop transformation methods which lead to a transgenic line which is insensitive to transposase, or in which the insertion is insensitive to transposase.

In order to stabilise the genetic insert, it is possible to provide three or more repeats, and locate the insert between two repeats in the same orientation. It follows that the number of transposable elements is equivalent to the number of repeats in one orientation multiplied by the number of repeats having the opposite orientation. Exposing the construct to transposase will result in all of the possible transposable elements being obtained, including a level of excision of the entire length of the transposon containing the gene of interest, which can then be inserted into the genome. The resulting insertion can then lose the shorter length which does not contain the insert by further, or the same, transposase activity. This shorter length is a transposable element flanked by inverted repeats. Once removed, it leaves the desired genetic insert in place, but with only one repeat, thereby disabling the ability of transposase to excise the genetic insert. This process is subject to extremely low success rates, however, as the transposase will usually generate the shortest length transposable element.

Thus, transposable elements with repeated ends can potentially utilise any of their repeated ends for transposition or excision. It is therefore possible to obtain transposition using a first distal end, followed by excision using another, more proximal end, leaving a fragment behind. This scheme is illustrated in Figure 1.

In Figure 1, triangles A-C represent the ends of the transposon, for example, the short inverted repeats at the ends of a class II element such as *piggyBac*. Transposition can occur between any two opposed triangles: in this case, A-B and A-C. Flanking transposon DNA may also be included, see below. Appropriate target site sequence, for example, TTAA or (A/T)N(A/T)TTAA(A/T)N(A/T) is also assumed to be included in these examples. Proximal and distal are relative to the 5' end. The construct of Figure 1A is introduced into cells or embryos, for example, by microinjection, transfection, or ballistic or other methods, with a

suitable transposase helper, for example, helper plasmid, RNA, transposase protein or integrated transposase source, also known as a jumpstarter.

A transformant containing the integrated product must then be selected, as shown in Figure 1B.

The selected transformant is then exposed to a suitable source of active transposase, for example, helper plasmid, RNA, transposase protein or an integrated transposase source, to generate the specific excision product shown in Figure 1C. The resulting product is substantially or completely stable to further exposure to active transposase, relative to the starting construct.

In the above example, two integration events are possible in the initial transformation step, then two excisions are possible once the desired integrant has been obtained. The alternative integration utilises the proximal 3' end, resulting in the insertion of a smaller transposon than desired, lacking the DNA of interest; the alternative excision utilises the distal 3' end, resulting in the complete excision of the construct and hence excision of the DNA of interest. Insect transformation is an inefficient process, so it is desirable to optimise the efficiency of this step. With suitable markers, even inefficient or rare excision may be acceptable, as an integrated transposase source can be used, so that it is possible to score large numbers of insects without requiring microinjection.

It is, therefore, highly desirable to maximise the efficiency of the first step, so as to provide the greatest possible number of initial transformants obtained by microinjection, for example, and obtain as many full-length insertions as possible and to maximise the ratio of full-length to short insertions.

Another available option for insect transformation involves adding another mobile element near or at one end of the transposon and rely on imprecise excision of this element to stabilise the insertion by deleting one end. This is an inherently unsatisfactory method and has never been done.

Another method for generating transgenics without flanking transposon DNA is well known in *Drosophila*, though not for any other insect. This is the homologous recombination system of Rong and Golic (Rong, Y., and Golic, K. (2000), Science *288*, 2013-2018; Rong, Y.,

and Golic, K. (2001), Genetics *157*, 1307-1312). Essentially, the contents of a transposon insert are moved to another, predetermined chromosomal location. The new insertion need not have any transposon DNA, though it does have to have a target site for a site-specific recombinase, such as Flp/FRT. As originally described, this insertion is always associated with a large direct repeat. The main limitation of this scheme is the highly variable, and generally very low, rate at which the new homology-based insertions are recovered. It also requires several enzyme activities to be available, such as by prior establishment of transgenic lines, for example FLP and I-SceI. The main use of this system is to generate knock-outs or other targeted modifications to specific genes or sequences.

What is required is a simple system with enhanced rates of initial transformation with a desired gene or nucleotide sequence.

The inverted repeat sequences of piggyBac transposons, for example, are associated with short, external TTAA sequences, which generally correspond to the insertion site. The inverted repeats are also associated with internal stretches of DNA. These internal stretches of DNA can be deleted to leave a minimal repeat, so that the repeats simply flank the desired DNA to be inserted. Transposons created in this manner are capable of excision and plasmid-to-plasmid transposition, and can do so with frequencies similar to those of constructs containing more internal piggyBac sequence (Elick et al., 1997, *supra*). However, such minimal piggyBac elements are not capable of efficient germline transformation, giving transposition frequencies approximately 20x lower than more complete elements.

It has also been established that transposable activity is retained if part of the repeat is deleted, but that the resulting insert can no longer be excised by transposase activity at the truncated repeat.

It has now been found that it is possible to provide a transposon with three or more inverted repeats of which a middle repeat is a minimal repeat, such a transposon providing significantly enhanced levels of initial transformation.

Thus, in a first aspect, the present invention provides a transposable element comprising at least three inverted repeats, at least one of which is inverted in relation to the others, wherein at least one non-terminal repeat is a minimal repeat.

It will be appreciated the greatest length of a transposon is the distance between, and including, the two opposing repeats farthest from each other. Other, shorter transposons can then be defined within the length of the longest transposon by the presence of other repeats. It is at least one of these non-terminal, or internal, repeats that are preferably minimal repeats.

As used herein, the term 'minimal repeat' applies to the highly conserved repeat sequences observed to be required for the activity of a given transposase. The piggyBac transposon, for example, has a 32bp terminal inverted repeat interrupted by a 4bp insertion at the 5' end and a 31bp insertion at the 3' end. This can be considered as two pairs of inverted repeats, one of 13bp and another of 19bp, these two being separated by 4bp at the 5' end and 31 bp at the 3' end. The minimal repeat, in this instance, then comprises a 32bp repeat with a 4 or 31 bp insertion at the 5' and 3' ends respectively.

It is generally preferred to provide the flanking sequences commonly associated with the terminal repeats. In the case of piggyBac, this is preferably TTAA.

In general, transposases will be more effective at cutting out shorter sequences so that, where a transposon has one 5' repeat and two 3' repeats, for example, the most common transposon that will be observed transferring to another locus will be the shorter, formed by the 5' repeat together with the more proximal of the two 3' repeats. This preference is both inevitable, owing to the fact that the longer transposon will still be cut by the transposase, as well as being exacerbated by topological considerations, whereby repeats in closer proximity appear to be bound substantially better by transposases.

The present invention overcomes this problem by employing at least one minimal repeat internal to the longest transposon of the construct. This minimal repeat is utilised at only very low rates for the original insertion, thereby strongly biasing the initial reaction to generating the larger transposon. Any DNA of interest is located between the minimal repeat and a corresponding full length repeat in the same orientation.

It is possible that subsequent exposure to transposase will simply lead to excision of the entire sequence, in which case the organism is effectively unaltered from the original. The

alternative provides the DNA of interest in combination with one full length repeat, but no corresponding inverted repeat, so that the insert is no longer part of a transposable element.

It will be appreciated that minimal repeats may simply comprise the minimum repeat necessary to effect transposition, and be associated with none of the original intervening DNA between the repeats observed in piggyBac, for example. However, the present invention envisages using some of the internal sequence, although it is preferred to keep this to a minimum, as greater lengths will increase the transposition frequency of the minimal repeat, thereby diminishing the desirable bias discussed above. Accordingly, while it is preferred to keep internal sequence associated with the minimal repeat to zero nucleotides, it is possible to use up to 100 bases, for example, of the original sequence, but it is preferred to use 50, or less, and preferably 10 or less.

Correspondingly, for the full length sequences, there is no clearly established limit as to what constitutes full length. It is established, however, that between about 6 and 14% of the internal sequence of a naturally occurring transposon is sufficient to provide high levels of transposition frequency, so that the present invention generally prefers that 'full length repeat sequences' be associated with at least 5% of the original internal sequence of the transposon from which they are derived, with between 6% and 14% being preferred, and 8% to 12% being more preferred.

It will also be appreciated that the sequences with which the repeats are associated need not correspond completely to the original sequences found in the naturally occurring transposons, and that variation and sequence degeneracy are encompassed within the scope of the present invention. In particular, it is preferred that any original sequence associated with the repeats of the present invention have at least 70% homology with the corresponding natural sequence, more preferably at least 80%, more preferably at least 90%, and particularly 95% or above, especially 100%.

The transposons of the present invention may employ two or more minimal repeats, although two is the preferred maximum. Where two are used, then it is preferred that these both be internal and in opposite orientations. Where two smaller transposons flank the DNA of interest, this has the particular advantage of encouraging the full length transposable element to be incorporated into the genome and then losing the two smaller transposons, thereby leaving

simply the DNA of interest without any flanking repeats. This method is also provided herein, simply using full length repeats, as defined above.

In general, it will also be appreciated that efficiency of generating full length transposable elements for incorporation into the genome can be enhanced by providing additional full length repeats at either or both ends of the element, thereby helping to bias the reaction away from any internal minimal repeat.

In order to ensure that the resulting organism has been transformed in the manner desired, it is preferred to utilise appropriate markers. These may be used in any manner suitable to inform the skilled person as to the status of the transformant. For example, markers may be associated with the DNA of interest in order to demonstrate that the organism has successfully been transformed. Markers may be incorporated in the areas between repeats that are to be deleted, so that the initial transformant containing the full length sequence can be detected. Any suitable combination of markers may also be used.

It is generally preferred that markers be selectable, either positive or negative, and suitable examples are illustrated hereinunder.

The use of any transposable element is envisaged, but class II elements, such as Hermes, hobo, Minos, and mariner, are preferred, owing to their relatively high fidelity during transposition, and the *piggyBac* element, which is known to use the distal element of a repeated pair at relatively high frequency [Elick, *et al.*, (1997), Mol. Gen. Genet. *255*, 605-610], is particularly preferred.

It is preferred to use a minimal piggyBac end as the more proximal of the distal ends and a fully-functional piggyBac end as the distal end. This arrangement strongly biases the initial transformation step towards insertion of the desired longer transposon.

In an alternative embodiment, multiple copies of the 3' and/or 5' ends is provided, with minimal repeats preferably providing the inmost of any series of repeats in the same orientation. This increases the chance that one or more longer versions of the transposon are integrated in the initial transformation, as required. One version of this is illustrated diagrammatically in Figure 2.

The construct of Figure 2A is introduced into cells or embryos, for example, by microinjection, transfection, or ballistic or other methods, with a suitable transposase helper, for example, helper plasmid, RNA, transposase protein or an integrated transposase source.

A transformant containing the integrated product of Figure 2B is then selected, and exposed to a suitable source of active transposase, for example, helper plasmid, RNA, transposase protein or integrated transposase source. Transformants containing the desired specific excision product, as shown in Figure 2C, can then be selected.

While efficiency in the second, excision step is less critical, as large numbers of individuals can readily be screened, it is preferred that the excision preferentially removes the short transposon, rather than a long one, as frequently as possible. The present invention provides such a system. piggyBac elements with suitable deletions or mutations in their internal inverted repeat are competent for transposition but not for excision, or have reduced excision rates. Use of such a modified end as the distal end will therefore bias the excision reaction towards utilisation of the proximal end as a higher proportion of the total of excision events.

Thus, it is preferred to use, as a terminal repeat, a repeat having a deletion of no more than 50%, or mutation or inversion that disables no more than 50% of the repeat. It is preferred that such a compromised repeat be in the same orientation as the minimal repeat, where there is only one. These repeats are readily transposed, but are not readily excised after transposition, thereby biasing the excision reaction towards the minimal repeat. Where more than one minimal repeat is used, in opposing orientations, then more than one compromised repeat may also be used.

The compromised repeat is generally preferred to correspond to a full length repeat, other than in respect of the deletion or mutation.

Figure 3 illustrates a scheme involving two pairs of inverted repeats flanking the gene or DNA of interest, and how this may be used to generate an insert ultimately associated with no transposon repeats.

The construct of Figure 3A is injected with a suitable transposase helper, for example, helper plasmid, RNA, transposase protein or integrated transposase source.

A transformant containing an integrated product as shown in Figure 3B may then be selected, and exposed to a suitable source of active transposase, for example, helper plasmid, RNA, transposase protein or integrated transposase source.

Transformants containing either of the specific excision products shown in Figure 3C may then be selected. Further exposure to transposase may then be employed to obtain the final excision product shown in Figure 3D.

The final transgenic line has no transposon DNA whatsoever associated with the insertion, unless this has been deliberately incorporated into the DNA of interest. Since there is no known upper or lower limit for the length of transposition-competent piggyBac elements that would constrain the length of the DNA of interest, the present invention provides a method of very general utility for inserting DNA into genomes of cells or organisms. However, we generally prefer that the total length of the initial piggyBac transposon be in the range 3-25kb, as this corresponds approximately to the range of piggyBac transposons commonly used for germline transformation in insects.

There is no lower limit to the amount of DNA that can be inserted by the overall procedure, after the flanking transposons have been excised. The initial insertion will retain the target site specificity of the original element, such as TTAA for piggyBac, with some apparent preference for (A/T)N(A/T)TTAA(A/T)N(A/T). Precise excision of the elements will resolve this to a duplication of the TTAA, flanking the DNA of interest, which can be as short as a single nucleotide. In the event that zero nucleotides are inserted, only the TTAA duplication remains. The insertion of larger fragments is generally preferred.

A suitable example of a small insertion is a stop codon. Insertional mutagenesis using transposable elements is a well known method for genetic screens of various types. However, interpreting the phenotype may be complicated by the presence of the transposon, with its associated markers, promoters and other elements. A short insertion, such as TTAA or CTAG, which provides a total sequence between the piggyBac ends of TTAATTAATTAA and TTAACTAGTTAA, respectively, allows the insertion to be resolved to a TTAA duplication

with this four base insertion. TTAATTAATTAA and TTAACTAGTTAA, in these examples, provide stop codons in all three frames in both directions. An insert of zero base pairs provides a frame shift and a stop codon in two frames, although one of these is already present in the original TTAA.

The present invention may be used to provide a coding region, such as for a fluorescent protein or a transactivator protein, such as GAL4, GAL4delta, or tTA. An insertional mutant may then be resolved to a fusion protein. As this needs to be in frame and in the correct orientation to function, it is generally preferable to also provide a suitable IRES (internal ribosome entry site) element and coding region, to allow bicistronic expression, or two such in opposite directions to allow bicistronic expression for insertions of either orientation.

The present invention may also be used to insert a suitable target site for a site-specific recombinase, either with or without a marker and other sequences. Initial experiments with the non-deleted transposon may serve to establish the presence or absence of specific enhancer effects on a particular insertion site, and the viability and other properties of individuals carrying large insertions at this site. Suitable insertions could then be resolved by stepwise excision to the recombinase target site, either with or without a marker and other sequences. This could then be used as a docking site to enable the insertion of other DNA sequences at this pre-determined and partially pre-characterised position. This arrangement has significant benefits in allowing the insertion of heterologous DNA at a predetermined site. If desired, the site can be protected from nearby enhancers by flanking the recombinase target site with suitable insulator elements, for example scs and scs', or HS4.

The present invention may also be used to replace an existing transposon insertion with specific DNA in such a way that the insertion is substantially or completely stable to further exposure to active transposase. This can be performed by replacing the existing transposon with a composite transposon of the present invention. Methods for performing such a replacement have been described [*c.f.* Johnson-Schlitz, *et al., Mol Cell Biol* **13**, 7006-18 (1993); Cabrera, *et al., Genesis* **34**, 62-5 (2002); Sepp, *et al., Genetics* **151**, 1093-101 (1999); Lankenau, *et al., Mol Cell Biol* **16**, 3535-44 (1996); Gonzy-Treboul, *et al., Genes Dev* **9**, 1137-48 (1995); Heslip, *et al., Genetics* **138**, 1127-35 (1994); Gloor, *et al., Science* **253**, 1110-7 (1991)].

Minimal molecular markers are generally characteristic of the invention. It will be apparent to the person skilled in the art that PCR-based or other molecular analysis is capable of distinguishing each of the various possible forms at each stage. However, it is generally more convenient to provide at least one visible or selectable marker, and this is preferred. Suitable markers are well known to the person skilled in the art, and include: genes encoding fluorescent proteins, including GFP, DsRed and their mutant derivatives; genes encoding drug or antibiotic resistance, such as neomycin phosphotransferase, or hygromycin resistance; and markers capable of functionally complementing a visible mutant in the host organism, such as mini-*white*$^+$ or *rosy*$^+$ in *Drosophila*, white$^+$ in *Ceratitis capitata* or *Drosophila* cinnabar complementing *kw*^{w} in *Aedes aegypti*. It is particularly preferred that at least one such marker be associated with the section of DNA containing the DNA of interest, allowing the segment to be tracked through the integration and stepwise excision process.

Where the DNA of interest segment is associated with two repeated ends, then each of the two flanking elements may be marked. Double selection may be used to recognise the initial insertion and then track the stepwise excisions. Since the last step has no high-probability alternatives, individuals with the DNA of interest can be recovered in the final step even though they no longer have any associated visible marker. Thus, the present invention provides a novel method for recovering transgenics with no selectable marker.

Visible markers can generally be scored for or against, in other words are generally suitable for either positive or negative selection. Automated, or semi-automated systems for identifying, or identifying and separating individuals are available, and have the potential for screening very large numbers of individuals, for example in the transformation or excision steps. For the excision step, negative selection for the appropriate transposon is desirable, together with positive selection for the DNA of interest, or an associated marker, as appropriate for the precise scheme selected. Negative selection markers are also known that can be used to select against particular individuals carrying them; such a system can be conveniently used to screen, or to help to screen large numbers of individuals for excision of a specific region. Suitable negative selection markers include inducible or repressible lethals, one half of a biphasic expression system such as GAL4/UAS or tTA/tRE, if the other half can be provided separately, dominant temperature sensitive lethals such as the *Drosophila* DTSs, or synthetic ones such as a suitable toxic element operably linked to a heat-shock or other inducible or repressible promoter.

Multiple visible markers can be provided not only by using mutant derivatives of fluorescent proteins, for example, of GFP and DsRed, which are independently distinguishable based on their spectral properties (*c.f.* Horn *et al.*, 2002, *supra*), but alternatively by expressing the same or similar markers in different spatial or temporal patterns. For example, it is readily possible to distinguish between *Drosophila* Act5C-DsRed, which shows ubiquitous expression, particularly clear in the body of larvae and in the adult eye, and Act88F-DsRed, which shows in indirect flight muscles only, and is, therefore, visible in the thorax of late pupae and adults. These markers are, therefore, separated by both their spatial and their temporal patterns and insects carrying one, the other, or both can readily be distinguished.

Where the present invention relates to transposable elements with two repeated ends, though the initial transformation reaction and the first excision reaction have several potential outcomes, of which not all are desirable, the second excision reaction has only one excision product, and this is the desired one. Accordingly, it is possible not to mark one of the flanking transposons, to select by suitable methods the desired products of the first two reactions, and then to conduct the second excision reaction blind, by exposure to transposase, for example, at high concentration, or for several generations of exposure to a jumpstarter element, then identify the desired reaction product by molecular methods. It is also possible to perform both excision reactions in this way, selecting only for the presence of a marker in the intervening sequence. It is, therefore, possible to perform the entire sequence of reactions using only a marker in this region. This embodiment provides for particularly short flanking transposons, and thus for the maximum size of insert for a given initial composite transposon size.

It will be appreciated that though each step of the insertion sequence is described separately, in practice, exposure to transposase may induce several of these steps to occur within one generation, or without the intermediate being specifically identified. This is generally acceptable and may provide a faster route to the desired final structure, and may be encouraged or stimulated by the use of relatively high concentrations of transposase, or the use of hyperactive transposase or *cis*-acting sequences, if desired. It will be understood that suitable use of molecular and/or selectable markers can facilitate this process.

Transposases are necessary to the function of the present invention, but it is not critical as to how they are provided. They may be provided in any suitable manner, as detailed below, and

may be inherent in the cell, provided on plasmids or even provided within the element itself, although this is not preferred. The cell may also be dosed with the enzyme or mRNA encoding the enzyme, or even with a virus expressing the enzyme, for example.

Suitable transposase can be provided in any of several forms: injection or electroporation, for example, of a plasmid or RNA encoding the transposase, or of transposase protein itself. A transposase source may also be integrated into the target genome, to provide a 'jumpstarter' construct, or line. This is a preferred method for the excision steps; the jumpstarter element can be combined with the initial insertion by conventional breeding, or by making the primary transformant in the jumpstarter line. Excision products will then be generated spontaneously, without requiring further injection or electroporation. With suitable markers or molecular analysis, the desired products can readily be isolated, and separated from the jumpstarter, if required, by conventional breeding.

Although jumpstarters provided within the transposons of the invention are not generally preferred, it is possible to provide a coding sequence therefor in a shorter internal transposon that it is desired to excise. Thus, it is possible to generate a jumpstarter line through insertion of an autonomous element, followed by its resolution by self-catalysed excision to a non-autonomous element.

Claims:

1. A transposable element comprising at least three inverted repeats, at least one of which is inverted in relation to the others, wherein at least one non-terminal repeat is a minimal repeat.

2. An element according to claim 1, comprising DNA for insertion into a host genome located between the minimal repeat and a repeat having the same orientation as the minimal repeat.

3. An element according to claim 1 or 2, wherein DNA for insertion into a host genome is flanked by two pairs of opposing repeats excisable by a transposase *in situ* to leave said DNA without flanking repeats in the host genome.

4. An element according to claim 3, wherein each of the repeats bounding the DNA for insertion into a host genome is a minimal repeat.

5. An element according to any preceding claim, wherein at least one repeat distal to the DNA for insertion into a host genome in relation to a minimal repeat in the same orientation has an internal deletion or is otherwise compromised over up to 50% of its length, thereby reducing the frequency of excision by a transposase at that repeat.

6. An element according to any preceding claim having at least one genetic marker associated with an identifiable step in the transposition/excision process.

7. An element according to claim 6, wherein the marker is associated with the DNA for insertion into a host genome.

8. A transposable element comprising at least four inverted repeats, at least two of which are inverted in relation to the others, comprising DNA for insertion into a host genome located between two pairs of opposing repeats excisable by a transposase *in situ* to leave said DNA without flanking transposon-derived repeats in the host genome.

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9. A method for transforming an organism, especially an insect, comprising exposing replicative tissue of the organism to an element according to any preceding claim under

conditions effective to incorporate the element into the genome thereof and, subsequently or simultaneously therewith, providing conditions suitable to excise a transposon from the genome, and selecting an organism, or tissue therefor, comprising the DNA intended for insertion lacking repeats in at least one orientation.

10. An organism obtained in accordance with claim 9.

ABSTRACT

STABLE INTEGRANDS

A transposable element comprising at least three inverted repeats, at least one of which is inverted in relation to the others, wherein at least one non-terminal repeat is a minimal repeat, allows for greater efficiency of insertion of nucleotide sequences in the genome.

Fig 1.

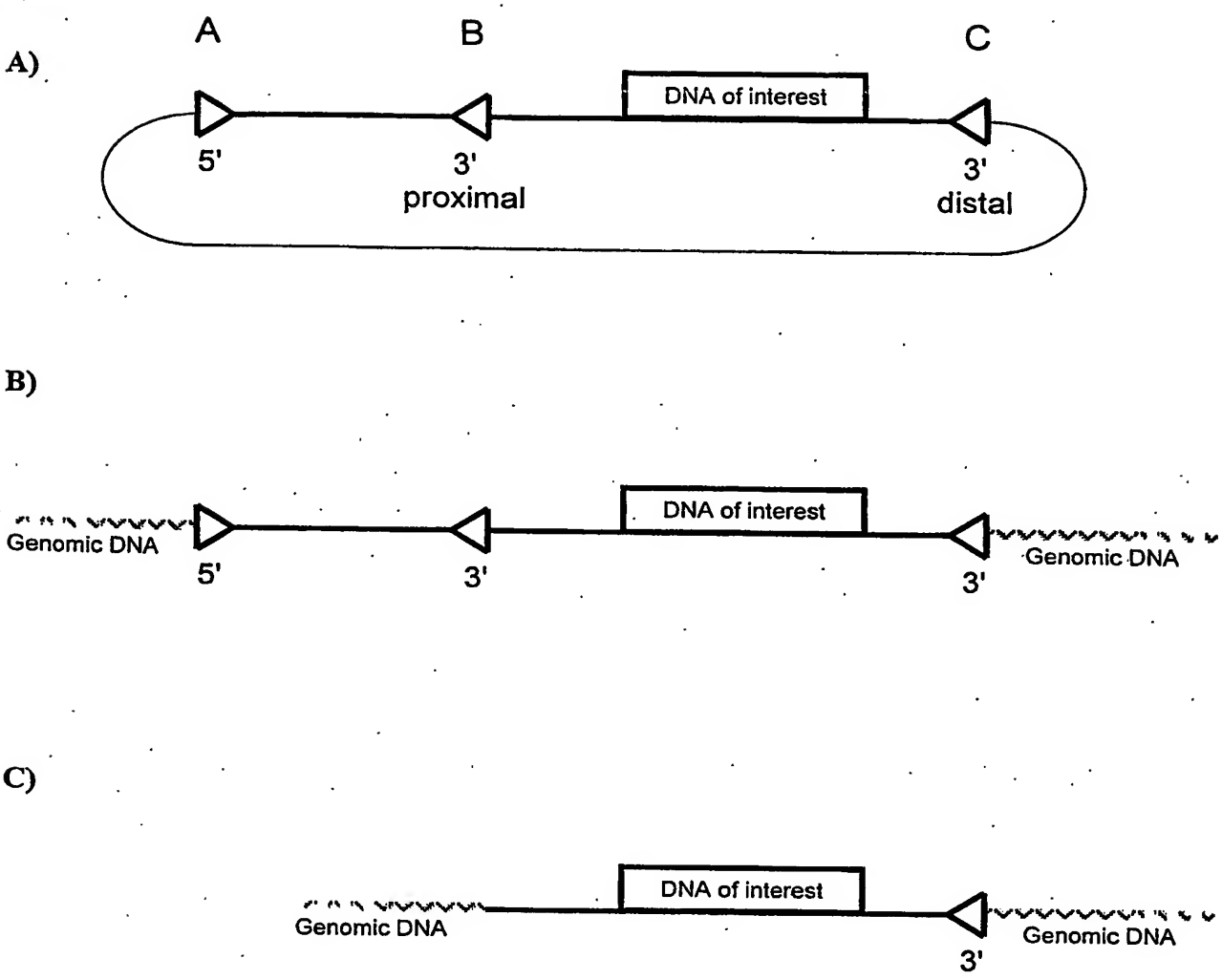
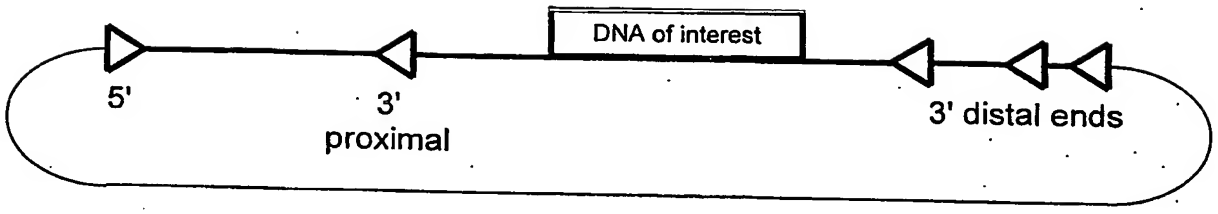
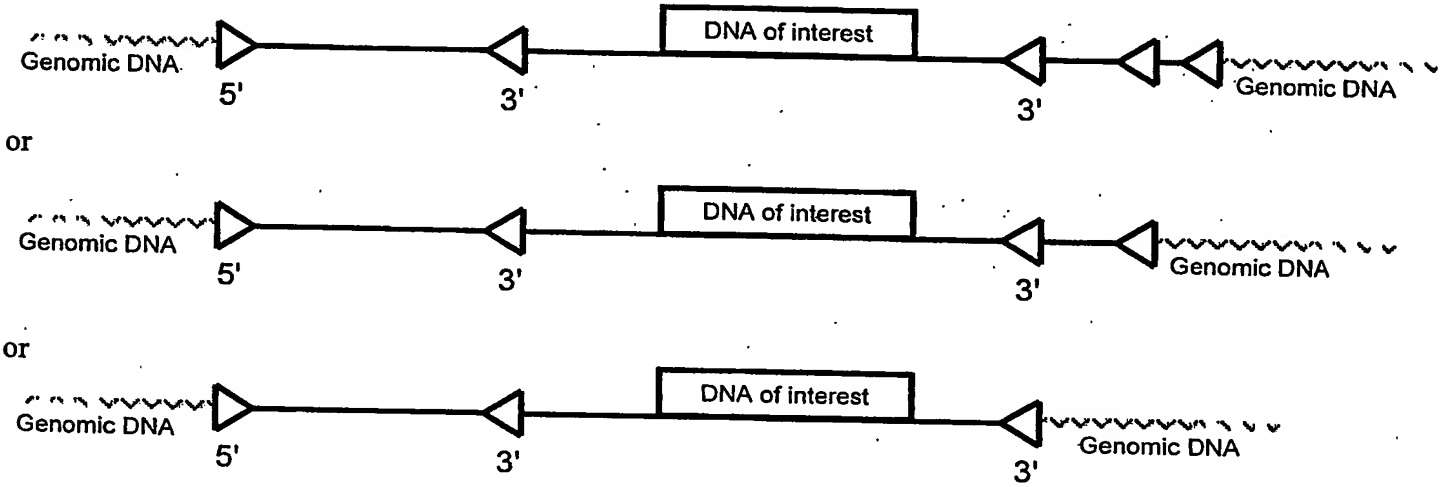

A)
A
B
C
DNA of interest
5'
3'
proximal
3'
distal
B)
Genomic DNA
DNA of interest
5'
3'
3'
Genomic DNA
C)
DNA of interest
Genomic DNA
3'
Genomic DNA

Fig. 1

A)


DNA of interest
5'
3'
proximal
3' distal ends

B)


Genomic DNA
DNA of interest
5'
3'
3'
Genomic DNA
or
Genomic DNA
DNA of interest
5'
3'
3'
Genomic DNA
or
Genomic DNA
DNA of interest
5'
3'
3'
Genomic DNA

C)

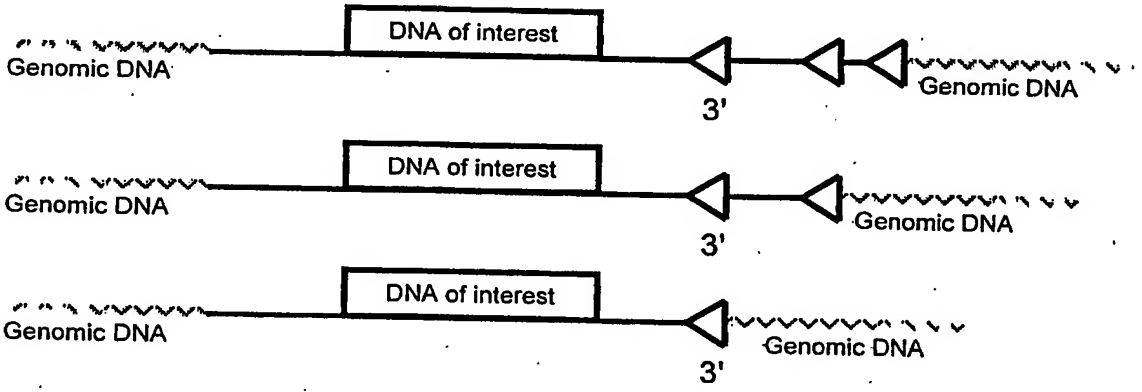

Genomic DNA
DNA of interest
3'
Genomic DNA
Genomic DNA
DNA of interest
3'
Genomic DNA
Genomic DNA
DNA of interest
3'
Genomic DNA

Fig. 2

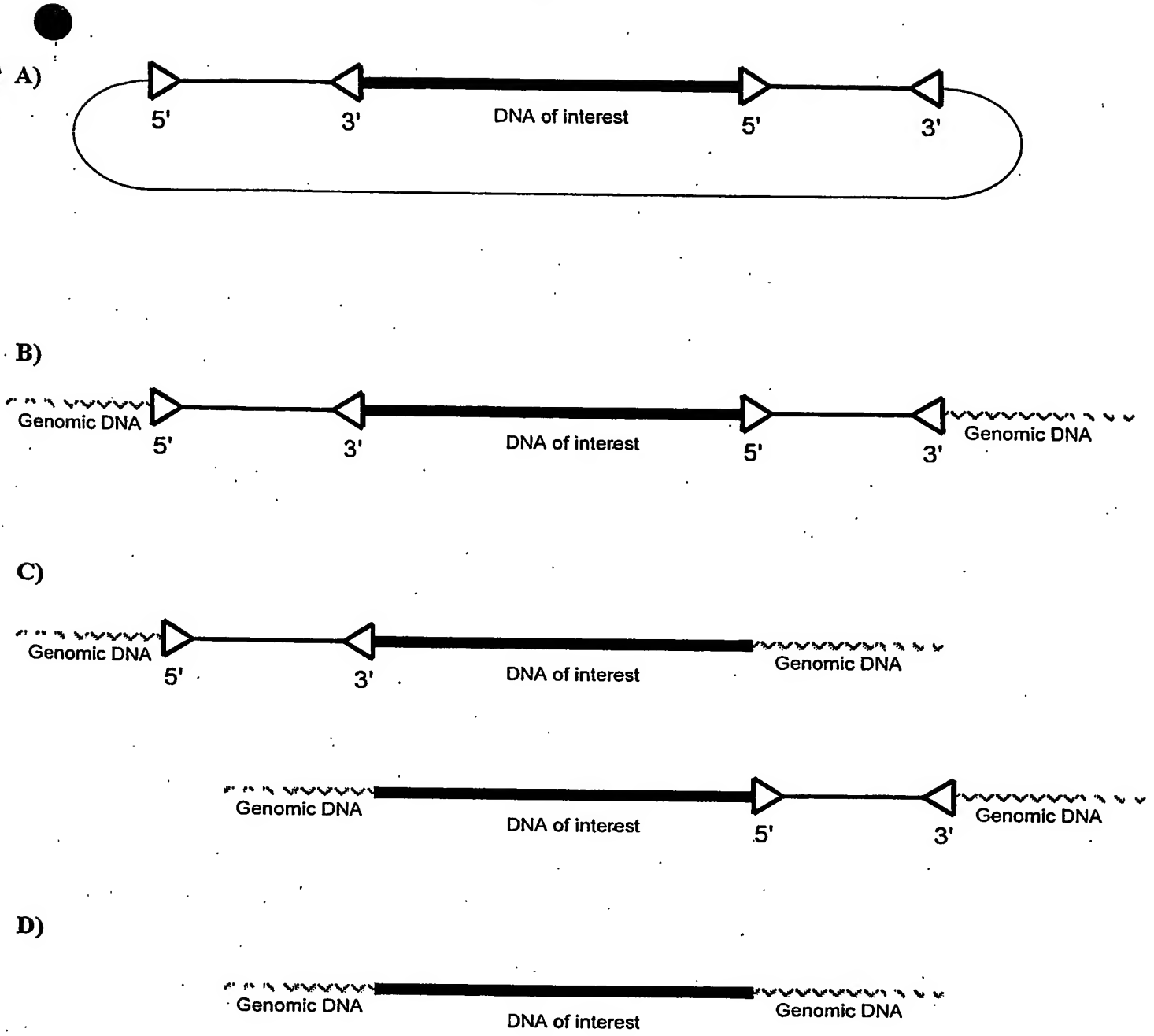

A)
5'
3'
DNA of interest
5'
3'
B)
Genomic DNA
5'
3'
DNA of interest
5'
3'
Genomic DNA
C)
Genomic DNA
5'
3'
DNA of interest
Genomic DNA
Genomic DNA
DNA of interest
5'
3'
Genomic DNA
D)
Genomic DNA
DNA of interest
Genomic DNA

Fig. 3

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